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Effects of Cholecystokinin-Octapeptide (CCK-8) on Food Intake and Gastric Emptying in Man

Reference #3

NORMA MUURAHAINEN, HARRY R. KISSILEFF,[1] ANDREW J. DEROGATIS AND F. XAVIER PI-SUNYER

Departments of Medicine, Psychiatry, and Radiology, Columbia University College of Physicians and Surgeons, and Obesity Research Center St. Luke's/Roosevelt Hospital Center, New York, NY 10025

MUURAHAINEN, N., H. R. KISSILEFF, A. J. DEROGATIS AND F. XAVIER PI-SUNYER. *Effects of cholecystokinin-octapeptide (CCK-8) on food intake and gastric emptying in man.* PHYSIOL BEHAV 44(4/5) 645–649, 1988.—Food intake and gastric emptying were measured simultaneously after cholecystokinin-octapeptide (CCK-8) and saline infusions in order to test the hypothesis that reduction in gastric emptying mediates the effect of CCK-8 on food intake. Each of twelve nonobese healthy men received intravenous infusions of CCK-8 and saline on separate nonconsecutive days after they had consumed 500 g of tomato soup tagged with technetium-99-DTPA. Intake of a test meal was measured 20 min after consumption of the soup while gastric emptying was simultaneously monitored by gamma emission scintigraphy of the soup. Food intake and gastric emptying of the soup were both significantly reduced by CCK-8 infusions in comparison to saline. There was a significant correlation between the amount of the test meal eaten and the amount of soup emptied during the period the test meal was being eaten, but not before the meal, only on days when CCK-8 was infused. Differences in intakes between days when saline was infused and days when CCK-8 was infused did not correlate with differences in gastric emptying of soup. These results suggest that CCK may amplify signals of satiety in proportion to the fullness of the stomach. Gastric emptying per se may not mediate the effects of CCK-8 on food intake.

Cholecystokinin Gastric emptying Human feeding Food intake Preloads Satiety

TWO lines of investigation led to this experiment. The first was the observation by Antin *et al.* (1) that cholecystokinin-octapeptide (CCK-8) was more effective in reducing intake in rats that had already eaten some food than in rats that were just beginning a meal. This led us to study the effects of CCK-8 in humans after they had eaten a preload (7,11). Later, we (9) directly tested the hypothesis that the preload was critical for the effectiveness of CCK-8 in reducing food intake in humans. Intake during CCK-8 infusion was only suppressed when a large 500 g (190 kcal) preload of tomato soup preceded the test meal but not when a smaller 100 g (38 kcal) preload preceded it. The use of the smaller preload ruled out the possibility that the preload was effective merely because of its taste or cognitive stimulation. Instead the amount of soup preload was critical. This result suggested that fullness of the stomach might be important in the effectiveness of the satiety-inducing effects of CCK.

The second line of research leading to this experiment was the observation of Moran and McHugh (8) that CCK was more effective in reducing food intake in monkeys when the animals were given a gastric preload of saline prior to an intravenous CCK infusion. Moran and McHugh (8) concluded that "CCK may act to reduce food intake indirectly through its capacity to produce gastric distension by retarding release of ingested food from the stomach." This hypothesis will be referred to as the "gastric mediation hypothesis."

Although Moran and McHugh (8) established the possibility that CCK could affect food intake through slowing gastric emptying, their work did not exclude the possibility that CCK might have independent effects on intake and on gastric emptying. In the present experiment we sought to obtain more direct evidence for the gastric mediation hypothesis by investigating whether a relationship existed between gastric emptying and food intake. To meet this objective, we measured gastric emptying of a tomato soup preload before and during test meals of macaroni and beef, when either saline or CCK-8 was infused intravenously. Our aim was to see whether differences in intake between saline and CCK-8 infusion conditions correlated with differences in gastric emptying when intake and empty-

[1]Requests for reprints should be addressed to Harry R. Kissileff, St. Luke's/Roosevelt Hospital Center, 114th St. and Amsterdam Ave., New York, NY 10025.

ing were measured in the same person, in the period immediately prior to the meal.

METHOD

Subjects

Twelve nonobese men were selected for this study by means of a taste test (7) and a test meal procedure designed to ensure that they liked the food and would eat what we considered a sufficient amount to constitute a meal (250 g). Participants had to meet the following criteria in order to enter the study: 1) rate both the test meal and the soup preload at least 6 on a 9 point acceptability scale (10) on a brief exposure taste test, 2) eat at least 250 g of the meal during an initial luncheon trial, 3) pass a physical examination, indicating that they were in good health, and 4) be within 15% of desirable weight for height (3). Informed consent was obtained, and the protocol was approved by the Institutional Review Board of St. Luke's/Roosevelt Hospital. The characteristics (mean±SD) of the participants were: age: 20.8 years ±3.3; height: 176.3 cm ±6.1; weight: 70.7 kg ±7.8; percent desirable (3): 102.5±8.3; acceptability of preload: 6.9±0.8; acceptability of test meal: 7.2±1.0.

Overall Design

After the participants were accepted into the study, they were given a 1-day adaptation trial followed by two test trials. On the adaptation trial saline was infused during the meal, but on the test trials either saline or CCK-8 was infused in a counterbalanced design so that half the participants received saline first while the other half received CCK first. All trials were conducted on nonconsecutive days with a maximum of three per week. On each trial, test meal intake and gastric emptying of a soup preload were measured simultaneously.

Daily Procedure

Participants came to the laboratory between 8:00 a.m. and 10:00 a.m., having fasted since midnight, to eat a standardized 300 kcal breakfast, consisting of a toasted English muffin, with 1½ pats of butter, and 250 g apple juice. The participant was then free to leave and returned to eat 2½ hr later. As indicated in Fig. 1, first a saline infusion (0.9%) was started in a forearm vein through a 23-gauge butterfly catheter and was infused continuously at the rate of 1.0 ml/min via a small (10×10×4 cm) portable (360 g) infusion pump. The participant was also positioned in front of a gamma scintillation camera for measurement of gastric emptying. The camera was fitted with a low energy, diverging hole collimator and was interfaced to a computer. The participant sat upright with his back against the camera and was therefore able to eat from a table in front of him. He was instructed to move as little as possible. Ten to 15 min were allowed for the participant to adapt to these circumstances before he received the preload. He remained seated for the remainder of the trial.

Twenty minutes before receiving the test meal (at time=0 in Fig. 1), the participant quickly consumed 500 g of hot tomato soup (Campbell, reconstituted with water according to the manufacturer's instructions, 0.38 kcal/g, approximately 130°F). Technetium-diethylenetriaminepentaacetic acid (99m-Tc-DTPA, 250 microcuries), a radiopharmaceutical, was added to the soup to serve as a marker for measurement of gastric emptying of the liquid phase. This radiopharmaceutical is useful for measuring gastric emptying because

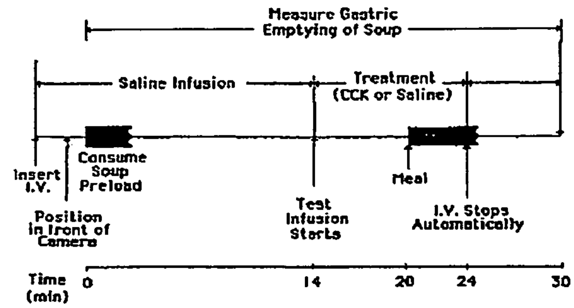

FIG. 1. Sequence of experimental procedures when participants returned for the luncheon meal. See text for explanation.

it is neither adsorbed to, nor adsorbed from, the gastrointestinal surfaces, and it has better aqueous phase stability than other commonly used technetium markers, e.g, Tc-99m sulfur colloid (4,13). The 99m-Tc-DTPA was freshly prepared each day using a commercial mixture (Syncor). Radioactive counts from the abdomen were recorded by gamma camera scintigraphy every 2 min for the first seven participants and every 30 sec for the rest.

Ten minutes after the participant had begun to consume the soup preload, the experimenter turned a switch on the infusion pump so that it began infusing from a second reservoir whose contents reached the blood stream 4 minutes later. The second reservoir provided either CCK-8 [total dose of 2.25 micrograms, Kinevac (Sincalide for injection), Squibb] or saline (0.9%) as the treatment infusion; the experimenter who served the meal was blind to its contents. Thus (as indicated in Fig. 1), the treatment infusion entered the bloodstream 14 min after the participant had begun to consume the soup. Both treatments were infused at 1.0 ml/min over a 10 min period, beginning 6 min before the test meal and ending 4 min into the test meal, regardless of when the participant stopped eating.

Six minutes after the onset of the treatment infusion [20 min after the beginning of the soup (see Fig. 1)], the participant was given a test meal of macaroni and beef (Stouffer, 1.16 kcal/g, heated to 130°F) to eat. He was instructed to eat until he had had enough and was satisfied as he would be with an ordinary meal eaten in a relaxed atmosphere. The meal was served on an eating monitor (5) which consisted of a table containing an electronic balance located under a panel on its surface. The monitor recorded the amount of food consumed every 3 sec. The resulting curve of cumulative food intake was fitted to a quadratic equation by least squares regression [SAS, 1982, GLM procedure (6,12)].

Data Analysis

The two main dependent variables in this experiment were the amount of test meal eaten and the amount of soup emptied either during the 6-min "premeal treatment period" (from the time the treatment infusion reached the vein until the moment the participant began eating the test meal) or during the "meal period" (from the time the participant started eating the test meal until its conclusion—as indicated in Fig. 1). Only the emptying of the soup and not the emptying of the test meal itself was monitored, since the radionuclide was in the liquid phase of the soup.

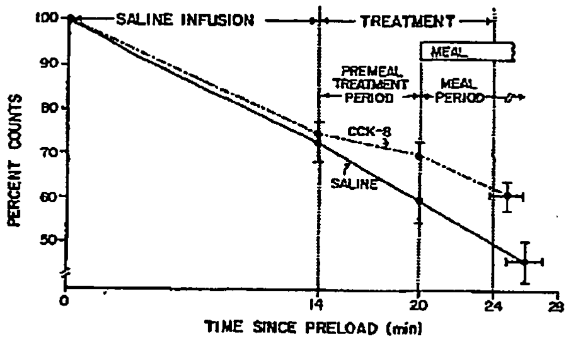

FIG. 2. Change in percent counts in stomach. Gastric emptying of radiolabeled soup. The saline infusion period began when the participant started consuming the preload and ended 14 min later when the test infusion reached the blood stream. The treatment period was the interval during which the test substance was infused. It was subdivided into the premeal treatment period (from the time the test substance entered the vein until the participant began eating the test meal) and the meal period (from the beginning of the meal until the participant stopped eating). Note that the infusion stopped at 4 min into the meal regardless of when the participant stopped eating. Data are expressed as percent of the initial peak counts obtained when the participant completed consumption of the soup.

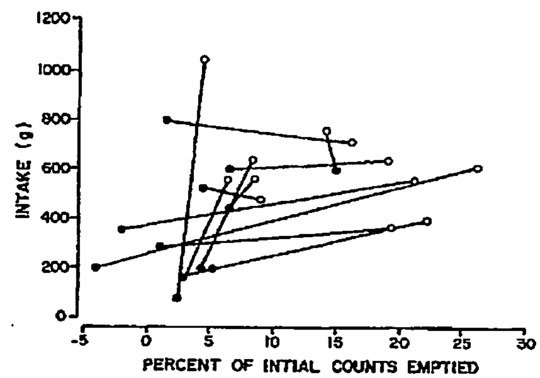

FIG. 3. Premeal treatment period—saline and CCK-8 day for each subject. Relation of test meal intake to gastric emptying expressed as the percent of initial peak counts emptied during the *premeal treatment period* (i.e., difference in percent remaining at the beginning and end of the premeal treatment period). The filled symbols show days on which CCK-8 was infused, and the unfilled show days on which saline was infused. Lines connect data from the same participant. Had there been a significant correlation of the differences in test meal intake, between CCK-8 and saline infusion conditions, with differences in amount of soup emptied before the test meal between CCK-8 and saline infusion conditions, the slopes would all have been parallel. There was no significant correlation of intake with emptying before the meal on either day nor was there a significant correlation of the differences in intake between saline and CCK-8 infusion conditions with the differences in soup emptied before the meal between the two treatment conditions.

In order to measure gastric emptying, the experimenter displayed the gamma emission intensity on a computer terminal and traced a region of interest, corresponding to the stomach. A computer count was then performed and the number of counts in the region of interest at each 2 min or 30 sec time point was expressed as a percent of the maximum number of counts. The percentage emptied during the premeal treatment period and the meal period was calculated by taking the difference between the percentages remaining at the beginning and at the end of each period.

Comparisons of variables between experimental conditions were made by means of paired, two tailed t-tests. Correlations between variables and between differences in variables between the experimental conditions were evaluated by means of Pearson product-moment correlations. The differences in variables were calculated by subtracting the variable's (e.g., intake or percent emptied) value on the CCK test day from its value on the saline test day for each subject. The SAS (12) statistical package was used to perform all computations.

RESULTS

Food Intake and Related Variables

Intake of the test meal was significantly less [240 g ±81 SED, paired $t(11)=2.97$, $p=0.013$] on days when CCK-8 was infused (362 g) than on days when saline was infused (602 g). Meal durations, however, were not significantly different between conditions (0.92 min ±0.60 SED, $p>0.05$), although they were somewhat less on CCK-8 days (4.93 min) than on saline days (5.85 min). The linear coefficient of the cumulative intake curve (6), which is equivalent to the initial rate of eating, was substantially, but not quite significantly less (49.7 g/min ±25.2 SED, $t=1.98$, $p=0.07$) after CCK infusions (81.5 g/min) than after saline (131.2 g/min). There was no significant difference (mean difference=0.26 g/min^2 ±3.75 SED g/min^2, $t=0.07$, $p=0.94$) in the quadratic coefficient, which is half the rate of deceleration of eating, between saline (−4.61 g/min^2) and CCK-8 (−4.34 g/min^2), indicating that the rate of deceleration of eating was not affected by the CCK-8 treatment.

Gastric Emptying

Gastric emptying was slower after CCK-8 than after saline infusions (see Fig. 2). However, at the conclusion of the 14-min saline infusion period following soup consumption, on days when participants later received CCK-8 (as a treatment infusion), the mean percentage of soup remaining in the stomach at 14 minutes was 75.2%. On days when they continued to receive saline as the treatment infusion, the mean percentage of soup remaining in the stomach at 14 min was 73.8%. Since the percentages emptied during the initial daily 14 min of saline (control period) were not significantly different between CCK and saline days, our measurement of emptying was relatively reproducible under control conditions. Assuming that all initial 500 ml of soup had entered the stomach, and that there was no significant absorption from, or secretion into, the stomach during this period, these percentages would correspond to remaining soup volumes of 376 ml and 369 ml, respectively.

During the 6-min premeal treatment period (Fig. 2), on days when saline was the treatment infusion, the percent remaining continued to drop by 14% (corresponding to a 70 ml decrease), reaching 61% by the time of meal onset. However, on days when CCK-8 was infused, emptying almost completely stopped during the premeal treatment period

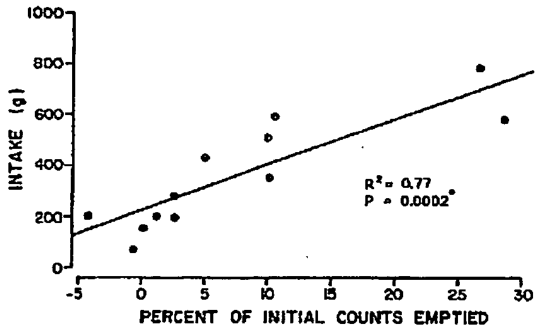

FIG. 4. Meal period—CCK-8 days. Relationship between intake of the test meal and gastric emptying of the soup during the *meal period* on days when CCK-8 was infused. Emptying is expressed as the difference in percent remaining in the stomach at the beginning of the meal and end of the meal (i.e., when participant stopped eating).

(Fig. 2), and only 5% of the initial 500 ml (or 25 ml) was emptied during this time. Although somewhat less soup was emptied by meal onset on days when CCK was infused, the difference in percent of soup emptied during the premeal treatment period between the two conditions (9% ±2.1% SED, or 45 ml—e.g., 70–25 ml) was not significant at the five percent level (paired t=2.05, p=0.064).

During the meal period, however, the amount of soup emptied was significantly different between conditions. When CCK-8 was infused, the amount remaining dropped to 62.5% of the initial amount by the end of the meal, while during saline infusions it dropped to 46% by the end of the meal (see Fig. 2). These amounts corresponded to 310 ml and 230 ml, respectively, of soup remaining in the stomach. The difference between them (26% ±5% SED or 80 ml) was significant (paired t=3.13, p=0.0096).

Relationship Between Gastric Emptying and Test Meal Intake

There was no correlation between the amount of test meal eaten and percentage of soup emptied during the premeal treatment period for the days on which the participants received saline nor for the days on which they received CCK-8. There was also no correlation of the differences in intake between CCK-8 and saline infusion conditions with the differences in emptying between CCK-8 and saline infusion conditions during the premeal treatment period (r^2=.03, p=0.59, see Fig. 3).

A similar absence of correlation was also seen between intake and emptying during the meal (as contrasted with the premeal treatment) period, on saline days. However (as shown in Fig. 4) there was a highly significant correlation (r^2=.77, p=0.0002) between intake and soup emptying during the meal on the days when CCK-8 was infused. The differences in intake between saline and CCK-8 infusion conditions did not correlate with the differences in gastric emptying between the saline and CCK-8 infusion conditions during the meal period (r^2=.26, p=0.09).

DISCUSSION

CCK-8 infusions, when compared with saline infusions, resulted in significant reductions in test meal intake and gastric emptying. However, we did not observe the predicted correlation of differences in gastric emptying with differences in intake between the CCK-8 and saline infusion conditions either before or during the meal. Instead the only significant correlation occurred between the amount eaten and the amount emptied during the meal, but not before the meal, on days when CCK-8 was infused. One possible explanation of the failure to see the expected correlation of differences in intake with differences in emptying during the premeal treatment period is that the length of the premeal treatment period was too short and the range of retardation of gastric emptying was too small for a correlation to emerge. However, since the short interval did result in reduction of intake in the present experiment, and since even shorter premeal treatment periods have previously resulted in consistent CCK-induced reductions in food intake (7, 9, 11), this explanation seems unlikely. It may be that gastric emptying per se plays no role in the control of intake.

On the other hand, the one correlation that we did see during the meal period is consistent with the recent observations of Barber and Burks (2). They showed that more electrical activity from the vagus nerve of a cat was recorded when CCK was infused and the stomach was distended than when it was not distended. Our results are consistent with their finding that CCK amplifies the signals of gastric distension to the brain. These signals may inhibit food intake.

The correlation between intake and emptying observed during the meal on days when CCK-8 was infused could be attributable to the common factor of meal duration, since meal duration is related to both the amount of emptying and the amount of intake. However, this possibility is lessened by the fact that the same argument would apply to the saline days during which no correlation was seen. Yet, it remains possible that the correlation occurred because the reduced food intake during CCK days widened the range of intake and duration of the period over which emptying was measured. If this explanation were correct, it would mean that the reduction in food intake had retarded gastric emptying, and not that a delay in gastric emptying had decreased intake.

The results on food intake reduction confirm our previous studies (7, 9, 11) which showed that food intake in humans was reduced by infusion of CCK-8. It is of interest that in the present study, as well as in one previous study (9), a different test meal was given, macaroni and beef, instead of yogurt-fruit drink given in the first two studies from our laboratory (7,11). Thus the phenomenon is robust across meal variation.

In conclusion, further investigation is required in order to determine whether the lack of correlation of the differences in intake with differences in emptying during the premeal treatment period was the result of too short a measurement period for emptying, or whether the robust correlation between intake and emptying, per se, during the meal period on days when CCK-8 was infused, represents a different mechanism of action for CCK-8 on gastric emptying than the gastric mediation hypothesis proposed by Moran and McHugh (8).

ACKNOWLEDGEMENTS

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